



Life Sciences Division

E-Newsletter March 31, 2008

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DOE scientific focus area notes

Low Dose Radiation Research

Coordinating Multi-Disciplinary Expertise

The LBNL Low Dose Radiation Research Program now constituted under the auspices of the Scientific Focus Area (SFA) funding paradigm will address the cell and molecular biology, human genetics and tissue physiology that determine responses to low-dose radiation. The proposed program represents an evolution of our long-standing program in low dose radiation research. Researchers at LBNL have pioneered studies of the fundamental mechanisms of homeostatic control and epithelial carcinogenesis mediated by the microenvironment and how these are affected by radiation. LBNL researchers have also characterized novel molecular mechanisms for coordinating DNA damage responses to oxidative DNA damage and their inducibility by low-dose radiation, identified new mechanisms of epigenetic regulation, and defined transcriptional response networks that are activated in response to low dose exposures. The new LBNL program focuses on coordinating this multi-disciplinary expertise to define the mechanisms and consequences of radiation response in tissues and complex biological systems in terms of endpoints that are relevant to regulatory decision making. We will study the impact of low dose radiation on three biological radiation-response processes: adaptive responses, non-targeted responses, and epigenetic regulation in relation to cancer, which is the clearest radiation-induced health effect and the endpoint that can be most easily translated into radiation regulatory models.

Mary Helen Barcellos-Hoff, 3/08

GTL-Genomics

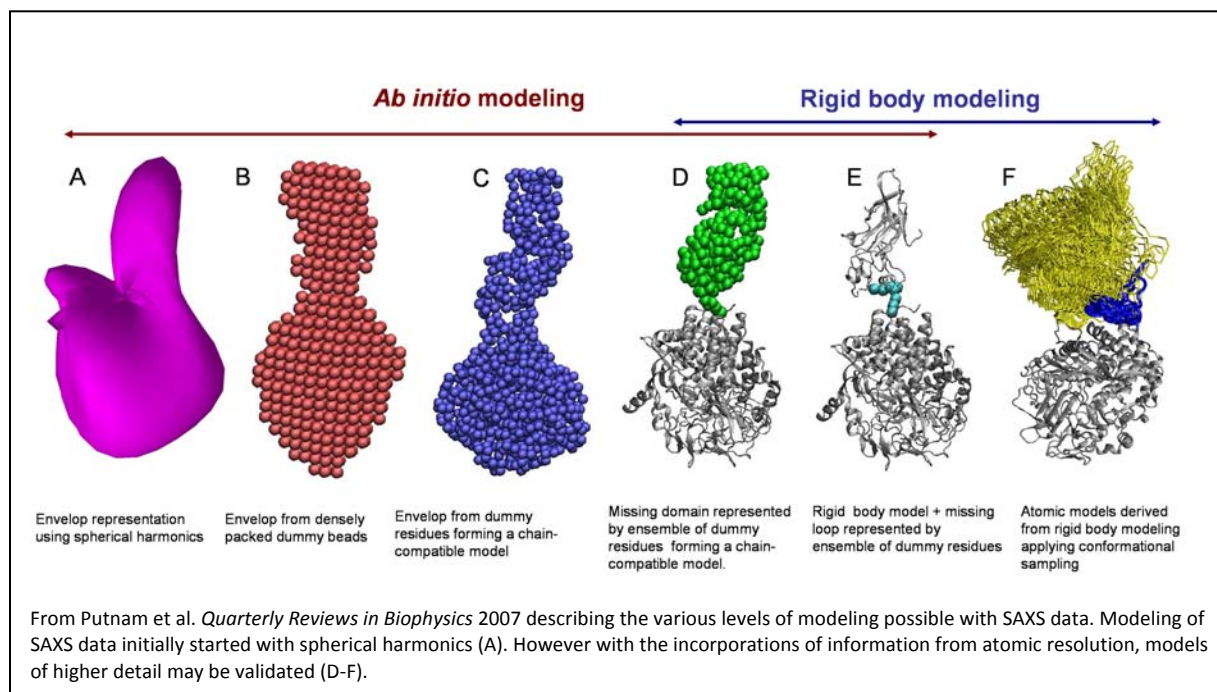
The SIBYLS Beamline, Leading Efforts for Proteomic Scale Structural Characterization

In a recent peer-reviewed methodological treatise focused upon data interpretation tools for combined SAXS-MX technologies, published in the journal Quarterly Reviews in Biophysics authors, Chris Putnam, Michal Hammel, Greg Hura and John Tainer, describe modern applications and implementations of small angle X-ray scattering (SAXS). This solution-based technique builds on the atomic resolution information generated in the last two decades of structural biology to efficiently characterize macromolecular complexes. The technology development and coupling of macromolecular crystallography and SAXS results have been funded largely through the DOE program project IDAT (Integrated Diffraction Analysis Technologies). Many examples have been highlighted within the review as well as in recent publications from users at the beamline (for examples see Shell et. al. Mol Cell May 2007, Corbett et al. Nat Struct Mol Bio. July 2007). The implementation of the SAXS pipetting robot has significantly improved the throughput of SAXS data collection and exploits the high brilliance light from the Advanced Light Source. Coupling analysis tools described in their review and the high throughput data collection, a pipeline has been developed for application of this technique.

This pipeline has been implemented to structurally characterize purified macromolecular complexes generated in the DOE funded collaborative project MAGGIE (Molecular Assemblies, Genes and Genomics Integrated Efficiently). MAGGIE is also supported by an innovated mass spectrometry component (recent publication by Northen et al Nature Oct 2007) and a bioinformatics component

(recent publication by Bonneau et al. Cell Dec 2007). Several publications of the structural analysis of MAGGIE complexes are underway. Given the large amount of data generated in this portion of the MAGGIE project, a web accessible utility (BioSis) has been developed and is available at www.bioisis.net. This is the first SAXS database available for the deposition of SAXS results and is intended to be for SAXS data what the Protein Data Bank is for crystallographic data.

Greg Hura/ John Tainer, 2/08



Nuclear Medicine

LBNL Facility for Crystal Growth

In a joint effort, the Lab's Life Sciences and Materials Sciences Divisions are establishing the LBNL Crystal Growth Facility. This facility will help fill a national need for crystal samples essential in the development of improved superconductors and nuclear detectors with applications in physics, biomedicine, and security. Using funding from DOE's Office of Basic Energy Sciences, DOE's Office of Nonproliferation, and the Department of Homeland Security Domestic Nuclear Detection Office, six crystal growth furnaces will be installed at the Berkeley Lab. These systems were specifically chosen for the rapid production of small test crystals under high purity conditions. Some of the furnaces can reach temperatures of 2400°C and can convert a sintered rod into a single crystal without the need of a crucible. Nuclear Medicine is one of the Lab's research programs that will be supported by this facility through the production of new semiconductor crystals for Single Photon Emission Computed Tomography (SPECT) and new scintillator crystals for Positron Emission Tomography (PET).

Stephen Derenzo, 3/08

Scientific news

SATB1 Protein Triggers Aggressive Breast Cancer

SATB1 is a nuclear protein well known for its crucial role in regulating gene expression during the differentiation and activation of T cells, making it a key player in the immune system. But SATB1 has now revealed a darker side: it is an essential contributing factor in the most aggressive forms of breast cancer. "In breast tumors, SATB1 reprograms the genome to change the expression of hundreds of genes, promoting tumor growth and metastasis," says **Terumi Kohwi-Shigematsu**, with Berkeley Lab's Life Sciences Division. [Full story] <http://www.lbl.gov/Science-Articles/Archive/LSD-SATB1.html>
Today at Berkeley Lab, 3/13/08

The above article was featured in Nature's email forecast of the March 13 issue. Here is the Nature write-up: "Geneticists have identified a gene that promotes aggressive breast cancer by altering the behaviour of more than 1,000 other genes within tumour cells. What's more, they find that knocking out this 'kingpin' gene causes the cancer cells to stop their runaway proliferation. The gene, called SATB1, already known to be expressed in breast tumours, is a key factor in the process of metastasis — the spread of cancerous cells to other locations in the body — report Terumi Kohwi-Shigematsu and co-workers in this week's Nature. In mouse models, they found that disrupting SATB1 stops cancer cells from dividing and spreading. Conversely, deliberately expressing this gene in cancer cells causes them to form very aggressive tumours. This is consistent with SATB1's normal role in the cell, as an 'organizer' of other genes, the researchers add. Aggressive tumours therefore form when this gene is overactivated, and the 'mob' of growth-promoting genes that it controls begins to run amok."

The SATB1 press release garnered a tremendous amount of media attention. Here are some samples of the requests that came in: Request for images to illustrate interview of Terumi Kohwi-Shigematsu (by reporter Roger Highfield), from Matthew Lidbury, picture editor, The Daily Telegraph; request for contact information and explanations from Michelle Cortez, editor, Bloomberg Network News SATB1, top 5 news story on Yahoo overnight (right up there with "Spitzer resigns," "Ferraro resigns," and "Mexican drug cartels"....); picked up by The BCN (BreastCancer.Net) News (online); picked up Medical News Today and MediLexicon (online); request for contact information and explanation from Miranda Hitti, medical writer, WebMD.com; request for images from Cilene Pereira, reporter, Istoe (weekly news magazine, Brazil); request for contact information on Hye-Jung Han from Chaerim Hah, staff reporter, Yonhap News Agency (Korea).

Berkeley Lab Weekly Media Report, 3/10 - 3/14/08

Breast Cancer Retreat Hosted by Joe Gray

Joe Gray, Associate Lab Director and leader of the UC San Francisco Breast Oncology program, invited the Lab community to attend a retreat on breast cancer research on January 31 and February 1, 2008. The 2008 Breast Oncology Program Retreat focused on population sciences, experimental therapeutics, computational biology, and epigenetics. The retreat included a juried poster session. The event was held at the Jewish Community Center of San Francisco.

Today at Berkeley Lab, 1/28/08

Study Shows Irregular Exercise May Add Pounds

The consequences of quitting exercise may be greater than previously thought, according to a new study that determined that the weight gained during an exercise hiatus can be tough to shed when exercise is resumed at a later date. The study, conducted by Berkeley Lab life scientist **Paul Williams**, found that the key to staying trim is to remain active year-round, year after year, and to avoid seasonal and irregular exercise patterns. Most of all, don't quit. Failure to do so may be a contributing factor in the nation's obesity epidemic. [Full Story] <http://www.lbl.gov/Science-Articles/Archive/LSD-irregular-exercise.html>
Today at Berkeley Lab, 2/4/08

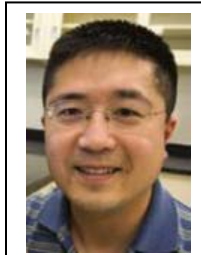


Paul Williams was interviewed about his diet studies by Jane Bianchi, associate health editor of Family Circle, Julliane Silveira, a reporter from the Brazilian newspaper "Folha de S. Paulo" and by a reporter for a Polish weekly magazine called "Wprost".
Berkeley Lab Weekly Media Report, 3/3 - 3/7/08

Some Gene Binding May Mean Nothing

Biologists are developing ever more sophisticated means to characterize molecular interactions in living systems. But many of these interactions may be functionally irrelevant, says a new study in the online journal *PLoS Biology*, led by Michael Eisen, **Mark Biggin**, Xiao-Yong Li, and Stewart MacArthur of Berkeley Lab's Genomics Division. Transcription factors that choreograph early development in the fruit fly bind to a surprisingly wide array of genes, but much of this binding has no effect on gene expression, the researchers found. The work was carried out as part of a broader collaboration by the Berkeley Drosophila Transcription Network Project (BDTNP) in which **several Life Sciences Division researchers** participate. [Full Story] <http://www.lbl.gov/Science-Articles/Archive/sabl/2008/Feb/genome-mystery.html>
Today at Berkeley Lab, 2/15/08

Technology Helps Assess Nanomaterial's Affect



Frank Chen

There are plenty of scare stories about health problems caused by nanotechnology, but where does the truth lie? **Frank Chen**, with Berkeley Lab's Life Sciences Division, has developed a way to predict and evaluate the effects of exposure to a particular nanomaterial on human skin cells. Once the skin cells have been exposed, Chen uses computerized image analysis to see whether the cells are dying, and genome analysis to see which genes have been switched on or off. [Full Story] <http://www.newscientist.com/blog/invention/2008/02/toxic-onions.html>
Today at Berkeley Lab 2/21/08

Life Scientist Comments on Rotavirus Milestone

The determination of high-resolution structures of large multiprotein complexes by cryo-EM just took an important step forward with the publication in PNAS of a structure of the icosahedral "inner capsid particle" of human rotavirus by a research team at Brandeis University and Harvard. In a Commentary

on this work published in the Feb. 12 issue of PNAS, Life Sciences Division scientist **Robert Glaeser** notes that the ability to build an atomic model of the viral proteins into the cryo-EM density map is at least as good as it is for the 0.38 nm-resolution X-ray crystallographic map to which it is compared. Data from images of more than 8000 viral particles were averaged in order to overcome the shot-noise limitations associated with recording the images at electron exposures that were low enough to avoid destroying the protein structure. By taking advantage of the 60-fold icosahedral symmetry and the additional 13-fold non-icosahedral symmetry of this particle, the authors were able to merge data from over 6.5 million copies of the viral proteins. Earlier studies, such as the structure determination of tubulin a decade ago by LBNL scientists **Eva Nogales** and **Kenneth Downing**, had achieved comparable resolution by averaging data from images of two-dimensional protein crystals. Subsequent work by others had achieved the same milestone by averaging data from images of well-ordered helical arrays of proteins.

As Glaeser points out, the significance of this new work is that images of viral particles were aligned, and their individual orientations in space were determined, by software tools identical to those used to align images of completely asymmetric single particles. The next step, truly a Grand Challenge in structural biology, may be to use automation of the whole process to make it practical to average data from millions of asymmetric particles, bypassing the need to either crystallize the proteins of interest or to work just with objects that occur in nature as highly symmetric particles. Alternatively, fundamental investigation into the reasons why the signal in cryo-EM images is so much weaker than it is in the scattered electron wave may lead to a 3-fold or greater improvement in the signal level in such images. If so, the number of particles that would have to be averaged would drop from the daunting level of ~ 5 million to a much more tractable level of about ~500,000. As Glaeser concludes in allusion to a poem by Robert Frost, cryo-EM still has promises to keep and miles to go before it can sleep. His full commentary can be found here: PNAS February 12, 2008 vol. 105 no. 6 1779–1780, <http://www.lbl.gov/today/2008/Feb/27-Wed/glaeser.pdf>
Robert Glaeser, 2/08; Also in Today at Berkeley Lab, 2/27/08



Accessing Accuracy of PHITS Code for Space Radiation

The Nuclear Fragmentation Team of **J. Miller**, **L. Heibronn**, and **C. Zeitlin** at LBNL has completed a physics survey comparing PHITS model predictions against experimental data for light, medium, and heavy beams. PHITS is an important code for both space and cancer therapy applications, and is under continuing development. This LBNL research team had earlier reported comparisons of PHITS to data from 12C beams. The deficiencies seen for medium and heavy beams are similar to each other, but a different set of problems was found when comparing to the 12C data. The team has two new publications towards significant improvements in the accuracy of the PHITS code. The first publication (in press in Physical Review C) is a report of the cross section measurements relevant to the space radiation problem, specifically to the transport of energetic heavy ions through matter. The measured cross sections are compared in detail to predictions from several theoretical models, with an emphasis on the Japanese code PHITS. The second publication (in press in Radiation Measurements), rather than reporting cross sections, presents the findings as fluences. The main difference between cross section and fluence data (aside from normalization) is that fluence data are not corrected for the secondary and higher-generation interactions that occur in the target. This makes fluence the more appropriate quantity when targets are thick. Comparisons are made to PHITS, which again addresses deficiencies in the nuclear interaction physics contained in that code.

Andrew Wyrobek, 2/08

International Research Collaboration on Lunar Regolith

The Nuclear Fragmentation Team of **J. Miller**, **L. Heilbronn**, and **Cary Zeitlin**, in collaboration with S. Guetersloh of NASA-JSC, completed several days of experiments with 290 MeV/u 10B and 11B beams at the NIRS HIMAC in Chiba, Japan during January 2008. The objective was to measure a depth-dose curve for simulated Apollo 17 lunar mare regolith. The regolith measurement is in support of proposed lunar habitat designs incorporating regolith as shielding. The depth-dose curve extracted from the data is consistent with model calculations indicating that, on average, the regolith, has shielding properties comparable to aluminum.

Andrew Wyrobek, 2/08

Annual Meeting of the National Council on Radiation Protection

Amy Kronenberg is on the program committee for the upcoming 44th Annual Meeting of the National Council on Radiation Protection, to be held April 13-15, 2008 in North Bethesda, MD. The theme for this year's annual meeting is Low Dose and Low Dose-Rate Effects and Models. Kronenberg is also co-chairing the session on Molecular, Cellular, Tissue and Animal Radiation Responses of Relevance to Radiation Protection. A key feature of this meeting will also be the session on low dose-radiation effects, regulatory policy and impacts on the public. DOE will be a key agency represented in this session with a perspective provided by Dr. Noelle Metting. **Andrew J. Wyrobek** and **Mary-Helen Barcellos Hoff** are invited speakers to this annual meeting.

Andrew Wyrobek, 2/08

American Statistical Association Meeting on Radiation and Health

Amy Kronenberg is the LBNL member of the organizing committee for the upcoming American Statistical Association meeting on Radiation and Health, to be held June 15-18, 2008 in Vail, CO. The kickoff session for this meeting was jointly organized by Kronenberg, Dr. Redpath (UC Irvine) and Dr. Brooks (Univ. Washington Tri-Cities) on the topic of basic radiobiology and epidemiology in the low dose region, with speakers discussing the influence on radiation carcinogenesis of factors including genomic instability, non-targeted/bystander effects, adaptive responses, environmental epigenetics and the genetic predisposition in the context of low dose mammographic exposures. DOE has supported this meeting. Kronenberg will serve as the discussant for this session.

Andrew Wyrobek, 2/08

Dietary Folate Intake Linked to Sperm Health

Healthy men who report lower levels of the nutrient folate in their diets have higher rates of chromosomal abnormalities in their sperm, according to a new study by UC Berkeley and Berkeley Lab researchers. Childbearing women are encouraged to include adequate folate levels in their diet, but the new findings provide evidence that what men eat may also affect reproductive health. **Andrew Wyrobek**, with Berkeley Lab's Life Sciences Division, was the study's co-principal investigator. [Full story] http://www.berkeley.edu/news/media/releases/2008/03/19_folatesperm.shtml

Today at Berkeley Lab, 3/20/08

Fly Research Bearing New Fruit in Battling Disease



Most people think of fruit flies as annoying little pests zipping around bananas or grapes on the kitchen counter. But to biologists, they're diamonds on the wing. And the field is undergoing a revival, attracting thousands of researchers who ground out almost 16,000 scientific papers in the last five years. "We are in a renaissance period," said **Susan Celniker**, a life scientist at Berkeley. "We now have fly models for all types of human diseases." [Full story]

<http://www.bnd.com/living/health/story/286459.html>

Today at Berkeley Lab, 3/24/08

Awards

Funded proposal: Mapping 3D Cell Wall Architecture and Composition

Source: Energy Biosciences Institute; Personnel: **Manfred Auer**, J. Liphardt, **K. Downing**, and **B. Parvin**

The program will develop an atlas of plant cell wall architecture and composition at nanometer resolution for dicots and monocots. It couples advanced sample preparation methods, tomographic electron microscopy, raman microscopy, and computational methods to construct a quantitative model of the 3D cell wall architecture for comparative analysis. This initiative provides the enabling technologies for multi scale imaging of structure and chemical composition for mapping heterogeneous spatial data.

Bahram Parvin, 3/08

Five Lab Scientists Get Mentor Awards

The Department of Energy's Office of Science has honored five Berkeley Lab researchers — **Manfred Auer** and **Danielle Jorgens**, (Life Sciences), Michael Martin (ALS), Howard Matis (Nuclear Science) and Margaret Torn (Earth Sciences) — as Outstanding Mentors for their work with students, coordinated through the Lab's Center for Science and Engineering Education (CSEE) last summer. "The role of mentors is absolutely essential in preparing the next generation of scientists and engineers," says Susan Brady, head of CSEE. "We hope more Lab scientists will consider serving as mentors this summer".

Today at Berkeley Lab, 3/10/08

Cancer Research Award Given to Life Scientists

The 2008 Team Science Award from the American Association for Cancer Research (AACR) has been won by a team led by **Daniel Pinkel** of the Life Sciences Division. Members include **Joe Gray** and **Damir Sudar** of Life Sciences and Robert Nordmeyer of Engineering, along with colleagues from UC San Francisco. The award honors the interdisciplinary team for their conception, implementation, and clinical application of pioneering comparative genomic hybridization techniques, which link tumor genomes to breast cancer outcomes. [Press Release] http://www.eurekalert.org/pub_releases/2008-03/aafc-lrh031108.php

Today at Berkeley Lab, 3/12/08

Cancer Society Gives Bissell Medal of Honor

Berkeley Lab Distinguished Scientist **Mina Bissell**, with the Life Sciences Division, has received the American Cancer Society's Medal of Honor. The award is the highest bestowed by the society. Bissell was recognized for her research on microenvironmental influences on gene expression and tissue specificity in normal and malignant breast tissue.

Today at Berkeley Lab, 3/14/08

Recent publications (selected)

Han HJ, Russo J, **Kohwi Y, Kohwi-Shigematsu T**. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature, 2008 Mar 13;452(7184):187 PMID: 18337816

Mechanisms underlying global changes in gene expression during tumour progression are poorly understood. SATB1 is a genome organizer that tethers multiple genomic loci and recruits chromatin-remodelling enzymes to regulate chromatin structure and gene expression. Here we show that SATB1 is expressed by aggressive breast cancer cells and its expression level has high prognostic significance ($P < 0.0001$), independent of lymph-node status. RNA-interference-mediated knockdown of SATB1 in highly aggressive (MDA-MB-231) cancer cells altered the expression of >1,000 genes, reversing tumorigenesis by restoring breast-like acinar polarity and inhibiting tumour growth and metastasis in vivo. Conversely, ectopic SATB1 expression in non-aggressive (SKBR3) cells led to gene expression patterns consistent with aggressive-tumour phenotypes, acquiring metastatic activity in vivo. SATB1 delineates specific epigenetic modifications at target gene loci, directly upregulating metastasis-associated genes while downregulating tumour-suppressor genes. SATB1 reprogrammes chromatin organization and the transcription profiles of breast tumours to promote growth and metastasis; this is a new mechanism of tumour progression.

Barcellos-Hoff MH. Cancer as an emergent phenomenon in systems radiation biology. Radiat Environ Biophys. 2008 Feb;47(1):33-8. Epub 2007 Nov 20. PMID: 18026977

Radiation-induced DNA damage elicits dramatic cell signaling transitions, some of which are directed towards deciding the fate of that particular cell, while others lead to signaling to other cells. Each irradiated cell type and tissue has a characteristic pattern of radiation-induced gene expression, distinct from that of the unirradiated tissue and different from that of other irradiated tissues. It is the sum of such events, highly modulated by genotype that sometimes leads to cancer. The challenge is to determine as to which of these phenomena have persistent effect that should be incorporated into models of how radiation increases the risk of developing cancer. The application of systems biology to radiation effects may help to identify which biological responses are significant players in radiation carcinogenesis. In contrast to the radiation biology paradigm that focuses on genomic changes, systems biology seeks to integrate responses at multiple scales (e.g. molecular, cellular, organ, and organism). A key property of a system is that some phenomenon emerges as a property of the system rather than of the parts. Here, the idea that cancer in an organism can be considered as an emergent phenomenon of a

perturbed system is discussed. Given the current research goal to determine the consequences of high and low radiation exposures, broadening the scope of radiation studies to include systems biology concepts should benefit risk modeling of radiation carcinogenesis.

Fernando Amat, Farshid Moussavi Luis R. Comolli, Gal Elidan, **Kenneth H. Downing** and Mark Horowitz
Markov random field based automatic image alignment for electron tomography Journal of Structural Biology , Volume 161, Issue 3, March 2008, Pages 260-275

We present a method for automatic full-precision alignment of the images in a tomographic tilt series. Full-precision automatic alignment of cryo electron microscopy images has remained a difficult challenge to date, due to the limited electron dose and low image contrast. These facts lead to poor signal to noise ratio (SNR) in the images, which causes automatic feature trackers to generate errors, even with high contrast gold particles as fiducial features. To enable fully automatic alignment for full-precision reconstructions, we frame the problem probabilistically as finding the most likely particle tracks given a set of noisy images, using contextual information to make the solution more robust to the noise in each image. To solve this maximum likelihood problem, we use *Markov Random Fields* (MRF) to establish the correspondence of features in alignment and robust optimization for projection model estimation. The resulting algorithm, called Robust Alignment and Projection Estimation for Tomographic Reconstruction, or RAPTOR, has not needed any manual intervention for the difficult datasets we have tried, and has provided sub-pixel alignment that is as good as the manual approach by an expert user. We are able to automatically map complete and partial marker trajectories and thus obtain highly accurate image alignment. Our method has been applied to challenging cryo electron tomographic datasets with low SNR from intact bacterial cells, as well as several plastic section and X-ray datasets.

Hudson SG, Garrett MJ, Carlson JW, Micklem G, **Celniker SE**, Goldstein ES, Newfeld SJ. Phylogenetic and genomewide analyses suggest a functional relationship between kayak, the *Drosophila* fos homolog, and fig, a predicted protein phosphatase 2c nested within a kayak intron. Genetics. 2007 Nov;177(3):1349-61. PMID: 18039871

A gene located within the intron of a larger gene is an uncommon arrangement in any species. Few of these nested gene arrangements have been explored from an evolutionary perspective. Here we report a phylogenetic analysis of kayak (kay) and fos intron gene (fig), a divergently transcribed gene located in a kay intron, utilizing 12 *Drosophila* species. The evolutionary relationship between these genes is of interest because kay is the homolog of the proto-oncogene c-fos whose function is modulated by serine/threonine phosphorylation and fig is a predicted PP2C phosphatase specific for serine/threonine residues. We found that, despite an extraordinary level of diversification in the intron-exon structure of kay (11 inversions and six independent exon losses), the nested arrangement of kay and fig is conserved in all species. A genomewide analysis of protein-coding nested gene pairs revealed that approximately 20% of nested pairs in *D. melanogaster* are also nested in *D. pseudoobscura* and *D. virilis*. A phylogenetic examination of fig revealed that there are three subfamilies of PP2C phosphatases in all 12 species of *Drosophila*. Overall, our phylogenetic and genomewide analyses suggest that the nested arrangement of kay and fig may be due to a functional relationship between them.

Li XY, Macarthur S, Bourgon R, Nix D, Pollard DA, Iyer VN, Hechmer A, Simirenko L, Stapleton M, Hendriks CL, Chu HC, Ogawa N, Inwood W, Sementchenko V, Beaton A, Weiszmam R, **Celniker SE**, **Knowles DW**, Gingeras T, **Speed TP**, Eisen MB, **Biggin MD**. Transcription Factors Bind Thousands of Active and Inactive Regions in the Drosophila Blastoderm. PLoS Biol. 2008 Feb 12;6(2):e27 PMID: 18271625

Identifying the genomic regions bound by sequence-specific regulatory factors is central both to deciphering the complex DNA cis-regulatory code that controls transcription in metazoans and to determining the range of genes that shape animal morphogenesis. We used whole-genome tiling arrays to map sequences bound in Drosophila melanogaster embryos by the six maternal and gap transcription factors that initiate anterior-posterior patterning. We find that these sequence-specific DNA binding proteins bind with quantitatively different specificities to highly overlapping sets of several thousand genomic regions in blastoderm embryos. Specific high- and moderate-affinity in vitro recognition sequences for each factor are enriched in bound regions. This enrichment, however, is not sufficient to explain the pattern of binding in vivo and varies in a context-dependent manner, demonstrating that higher-order rules must govern targeting of transcription factors. The more highly bound regions include all of the over 40 well-characterized enhancers known to respond to these factors as well as several hundred putative new cis-regulatory modules clustered near developmental regulators and other genes with patterned expression at this stage of embryogenesis. The new targets include most of the microRNAs (miRNAs) transcribed in the blastoderm, as well as all major zygotically transcribed dorsal-ventral patterning genes, whose expression we show to be quantitatively modulated by anterior-posterior factors. In addition to these highly bound regions, there are several thousand regions that are reproducibly bound at lower levels. However, these poorly bound regions are, collectively, far more distant from genes transcribed in the blastoderm than highly bound regions; are preferentially found in protein-coding sequences; and are less conserved than highly bound regions. Together these observations suggest that many of these poorly bound regions are not involved in early-embryonic transcriptional regulation, and a significant proportion may be nonfunctional. Surprisingly, for five of the six factors, their recognition sites are not unambiguously more constrained evolutionarily than the immediate flanking DNA, even in more highly bound and presumably functional regions, indicating that comparative DNA sequence analysis is limited in its ability to identify functional transcription factor targets.

Comolli LR, Spakowitz AJ, Siegerist CE, Jardine PJ, Grimes S, Anderson DL, Bustamante C, **Downing KH**. Three-dimensional architecture of the bacteriophage phi29 packaged genome and elucidation of its packaging process. Virology. 2008 Feb 20;371(2):267-77. Epub 2007 Nov 14 PMID: 18001811

The goal of the work reported here is to understand the precise molecular mechanism of the process of DNA packaging in dsDNA bacteriophages. Cryo-EM was used to directly visualize the architecture of the DNA inside the capsid and thus to measure fundamental physical parameters such as inter-strand distances, local curvatures, and the degree of order. We obtained cryo-EM images of bacteriophage that had packaged defined fragments of the genome as well as particles that had partially completed the packaging process. The resulting comparison of structures observed at intermediate and final stages shows that there is no unique, deterministic DNA packaging pathway. Monte Carlo simulations of the packaging process provide insights on the forces involved and the resultant structures.

Pluth JM, Yamazaki V, Cooper BA, **Rydberg BE**, Kirchgessner CU, **Cooper PK**. DNA double-strand break and chromosomal rejoining defects with misrejoining in Nijmegen breakage syndrome cells. DNA Repair (Amst). 2008 Jan 1;7(1):108-18. PMID: 17919995

NBS1-deficient cells exhibit pronounced radiosensitivity and defects in chromosome integrity after ionizing radiation (IR) exposure, yet show only a minor defect in DNA double-strand break (DSB) rejoining, leaving an as yet unresolved enigma as to the nature of the radiosensitivity of these cells. To further investigate the relationship between radiosensitivity, DSB repair, and chromosome stability, we have compared cytological and molecular assays of DSB misrejoining and repair in NBS1-defective, wild type, and NBS1-complemented cells after IR damage. Our findings suggest a subtle defect in overall DSB rejoining in NBS1-defective cells and uniquely also reveal reduced ability of NBS1-defective cells to rejoin correct ends of DSBs. In agreement with published results, one of two different NBS1-defective cell lines showed a slight defect in overall rejoining of DSBs compared to its complemented counterpart, whereas another NBS line did not show any difference from wild type cells. Significant defects in the correct rejoining of DSBs compared to their respective controls were observed for both NBS1-defective lines. The defect in DSB rejoining and the increased misrejoining detected at the molecular level were also reflected in higher levels of fragments and translocations, respectively, at the chromosomal level. This work provides both molecular and cytological evidence that NBS1-deficient cells have defects in DSB processing and reveals that these molecular events can be manifest cytologically.

Markstein M, Pitsouli C, Villalta C, **Celniker SE**, Perrimon N. Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes. Nat Genet. 2008 Mar 2 PMID: 18311141

A major obstacle to creating precisely expressed transgenes lies in the epigenetic effects of the host chromatin that surrounds them. Here we present a strategy to overcome this problem, employing a Gal4-inducible luciferase assay to systematically quantify position effects of host chromatin and the ability of insulators to counteract these effects at phiC31 integration loci randomly distributed throughout the Drosophila genome. We identify loci that can be exploited to deliver precise doses of transgene expression to specific tissues. Moreover, we uncover a previously unrecognized property of the gypsy retrovirus insulator to boost gene expression to levels severalfold greater than at most or possibly all un-insulated loci, in every tissue tested. These findings provide the first opportunity to create a battery of transgenes that can be reliably expressed at high levels in virtually any tissue by integration at a single locus, and conversely, to engineer a controlled phenotypic allelic series by exploiting several loci. The generality of our approach makes it adaptable to other model systems to identify and modify loci for optimal transgene expression.

Dong M, Yang LL, Williams K, Fisher SJ, Hall SC, **Biggin MD**, Jin J, Witkowska HE. A "Tagless" Strategy for Identification of Stable Protein Complexes Genome-wide by Multidimensional Orthogonal Chromatographic Separation and iTRAQ Reagent Tracking. J Proteome Res. 2008 Mar 13 PMID: 18336004

Tandem affinity purification is the principal method for purifying and identifying stable protein complexes system-wide in whole cells. Although highly effective, this approach is laborious and impractical in organisms where genetic manipulation is not possible. Here, we propose a novel

"tagless" strategy that combines multidimensional separation of endogenous complexes with mass spectrometric monitoring of their composition. In this procedure, putative protein complexes are identified based on the comigration of collections of polypeptides through multiple orthogonal separation steps. We present proof-of-principle evidence for the feasibility of key aspects of this strategy. A majority of *Escherichia coli* proteins are shown to remain in stable complexes during fractionation of a crude extract through three chromatographic steps. We also demonstrate that iTRAQ reagent-based tracking can quantify relative migration of polypeptides through chromatographic separation media. LC MALDI MS and MS/MS analysis of the iTRAQ-labeled peptides gave reliable relative quantification of 37 components of 13 known *E. coli* complexes: 95% of known complex components closely co-eluted and 57% were automatically grouped by a prototype computational clustering method. With further technological improvements in each step, we believe this strategy will dramatically improve the efficiency of the purification and identification of protein complexes in cells.

Groesser T, Chun E, **Rydberg B**. Relative biological effectiveness of high-energy iron ions for micronucleus formation at low doses Radiat Res. 2007 Dec;168(6):675-82. PMID: 18088180

Dose-response curves for micronucleus (MN) formation were measured in Chinese hamster V79 and xrs6 (Ku80(-)) cells and in human mammary epithelial MCF10A cells in the dose range of 0.05-1 Gy. The Chinese hamster cells were exposed to 1 GeV/nucleon iron ions, 600 MeV/nucleon iron ions, and 300 MeV/nucleon iron ions (LETs of 151, 176 and 235 keV/microm, respectively) as well as with 320 kVp X rays as reference. Second-order polynomials were fitted to the induction curves, and the initial slopes (the alpha values) were used to calculate RBE. For the repair-proficient V79 cells, the RBE at these low doses increased with LET. The values obtained were 3.1 +/- 0.8 (LET = 151 keV/microm), 4.3 +/- 0.5 (LET = 176 keV/microm), and 5.7 +/- 0.6 (LET = 235 keV/microm), while the RBE was close to 1 for the repair-deficient xrs6 cells regardless of LET. For the MCF10A cells, the RBE was determined for 1 GeV/nucleon iron ions and was found to be 5.5 +/- 0.9, slightly higher than for V79 cells. To test the effect of shielding, the 1 GeV/nucleon iron-ion beam was intercepted by various thicknesses of high-density polyethylene plastic absorbers, which resulted in energy loss and fragmentation. It was found that the MN yield for V79 cells placed behind the absorbers decreased in proportion to the decrease in dose both before and after the iron-ion Bragg peak, indicating that RBE did not change significantly due to shielding except in the Bragg peak region. At the Bragg peak itself with an entrance dose of 0.5 Gy, where the LET is very high from stopping low-energy iron ions, the effectiveness for MN formation per unit dose was decreased compared to non-Bragg peak areas.

Williams PT. Self-selection accounts for inverse association between weight and cardiorespiratory fitness. Obesity (Silver Spring). 2008 Jan;16(1):102-6. PMID: 18223620

BACKGROUND: Men and women who exercise regularly and who are physically fit tend to be leaner than those who are sedentary and not fit. Although exercise is known to attenuate weight gain and promote weight loss, there may also be a propensity for leaner men and women to choose to exercise vigorously (self-selection). Pre-exercise body weights have been shown to account for all the weight differences between fast and slow walkers, but seem to account for only a portion of the weight differences associated with walking distances. Whether these results apply to maximum exercise performance (i.e., cardiorespiratory fitness) as well as

to doses of vigorous exercise (metabolic equivalents >6) remains to be determined. **OBJECTIVE:** Assess whether the cross-sectional relationships of BMI to cardiorespiratory fitness and vigorous activity are explained by BMI prior to exercising. **METHODS AND PROCEDURES:** Cross-sectional study of the relationships between cardiorespiratory fitness (running speed during 10 km foot race) and vigorous physical activity (weekly running distance) to current BMI (BMI(current)) and BMI at the start of running (BMI(starting)) in 44,370 male and 25,252 female participants of the National Runners' Health Study. **RESULTS:** BMI(starting) accounted entirely for the association between fitness and BMI(current) in both sexes, but only a quarter of the association between vigorous physical activity levels and BMI(current) in men. In women, BMI(starting) accounted for 58% of the association between BMI(current) and vigorous activity levels. **DISCUSSION:** Self-selection based on pre-exercise BMI accounts entirely for the association found between fitness and BMI (and possibly a portion of other health outcomes).

Cools R, Gibbs SE, Miyakawa A, **Jagust W**, D'Esposito M. Working memory capacity predicts dopamine synthesis capacity in the human striatum. J Neurosci. 2008 Jan 30;28(5):1208-12. PMID: 18234898

Evidence from psychopharmacological research has revealed that dopamine receptor agents have opposite effects on cognitive function depending on baseline levels of working memory capacity. These contrasting effects have been interpreted to reflect differential baseline levels of dopamine. Here we demonstrate for the first time that working memory capacity as measured by listening span predicts dopamine synthesis capacity in the striatum, indicating that subjects with low working memory capacity have low DA synthesis capacity in the striatum, whereas subjects with high working memory capacity have high DA synthesis capacity in the striatum.

Marchetti F, Wyrobek AJ. DNA repair decline during mouse spermiogenesis results in the accumulation of heritable DNA damage. DNA Repair (Amst). 2008 Feb 16 PMID: 18282746

The postmeiotic phase of mouse spermatogenesis (spermiogenesis) is very sensitive to the genomic effects of environmental mutagens because as male germ cells form mature sperm they progressively lose the ability to repair DNA damage. We hypothesized that repeated exposures to mutagens during this repair-deficient phase result in the accumulation of heritable genomic damage in mouse sperm that leads to chromosomal aberrations in zygotes after fertilization. We used a combination of single or fractionated exposures to diepoxybutane (DEB), a component of tobacco smoke, to investigate how differential DNA repair efficiencies during the 3 weeks of spermiogenesis affected the accumulation of DEB-induced heritable damage in early spermatids (21-15 days before fertilization (dbf)), late spermatids (14-8dbf) and sperm (7-1dbf). Analysis of chromosomal aberrations in zygotic metaphases using PAINT/DAPI showed that late spermatids and sperm are unable to repair DEB-induced DNA damage as demonstrated by significant increases ($P < 0.001$) in the frequencies of zygotes with chromosomal aberrations. Comparisons between single and fractionated exposures suggested that the DNA repair-deficient window during late spermiogenesis may be less than 2 weeks in the mouse and that during this repair-deficient window there is accumulation of DNA damage in sperm. Finally, the dose-response study in sperm indicated a linear response for both single and repeated exposures. These findings show that the differential DNA repair capacity of postmeiotic male germ cells has a major impact on the risk of paternally transmitted heritable damage and suggest that chronic exposures that may occur in the weeks prior to fertilization because of

occupational or lifestyle factors (i.e., smoking) can lead to an accumulation of genetic damage in sperm and result in heritable chromosomal aberrations of paternal origin.

Chang, P.Y., K.A. Bjornstad, C.J. Rosen, S. Lin, **E.A. Blakely**. Particle radiation alters expression of matrix metalloproteases resulting in ECM remodeling in human lens cells. Radiat Environ Biophys. 46:187-194, 2007.

Blakely, E.A., P.Y. Chang. A Review of Ground-Based Heavy Ion Radiobiology Relevant to Space Radiation Risk Assessment: Part I. Cataracts and CNS Effects. Adv in Space Res. 40:1307-1319, 2007.

Blakely, E.A., P.Y. Chang. A Review of Ground-Based Heavy-ion Radiobiology Relevant to Space Radiation Risk Assessment. Part II: Cardiovascular and Immunological effects Adv. Space Res. 40:461-469, 2007.

Nikaniam M, **Blakely E.A.**, Bjornstad KA, Shu X, **Budinger TF, Forte TM**, Synthetic nano-low density lipoprotein as targeted drug delivery vehicle for glioblastoma multiforme, Int J Pharm. 328(1):86-94, 2007.

Thompson AC, **Blakely EA**, Bjornstad KA, Chang, PY, Rosen, CJ, and Schwarz RI, A Synchrotron-Based X-ray Exposure Station for Radiation Biology Experiments, Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment, Volume 582, Issue 1, 11 November 2007, Pages 226-228, Proceedings of the 14th National Conference on Synchrotron Radiation Research - SRI 2007.

Campisi J. Hot Topics Aging and Cancer Cell Biology, 2008. Aging Cell. 2008 Mar 10 PMID: 18331618

There is increasing support for the idea that aging and cancer are intimately connected by the activity of specific genes and the cellular responses to potentially oncogenic insults. This Hot Topics review discusses some recently published articles that shed light on both the commonalities - and intricacies - of the cancer-aging relationship. These articles reveal the expected complexities, but also surprising conservation, in mechanisms that link cancer and aging.

Brown LA, Kalloger SE, Miller MA, Shih IM, McKinney SE, Santos JL, Swenerton K, **Spellman PT, Gray J**, Gilks CB, Huntsman DG. Amplification of 11q13 in ovarian carcinoma. Genes Chromosomes Cancer. 2008 Mar 3 PMID: 18314909

Amplification at the 11q13 locus is commonly observed in breast, ovarian, head and neck, oral, and esophageal cancer. Studies of this region led to the identification of multiple amplicons containing several potential oncogenes including EMSY, PAK1, RSF1, and GAB2. Here, we investigate the amplification of the above four genes and their prognostic significance in histologically and clinically defined subsets of ovarian cancer. Amplification of all four genes was assessed by fluorescent in situ hybridization in tissue microarrays containing 538 clinically annotated ovarian carcinomas with 12 years of follow-up data. Overall, for the entire cohort, EMSY was amplified in 44 (16%) of 269 cases, PAK1 was amplified in 38 (15%) of 255 cases, RSF1 was amplified in 37 (12%) of 310 cases, and GAB2 was amplified in 41 (16%) of 255 cases. Amplification of EMSY, PAK1, RSF1, and GAB2 were all highly correlated with each other and

with a serous histology. Univariate survival analysis showed that tumors with EMSY and RSF1 amplification were associated with a significantly worse outcome. A molecular inversion probe array was then used to study the 11q13 amplicon in 33 high grade serous carcinomas. The core of the amplicon mapped to a 6-Mb region encompassing EMSY, PAK1, RSF1, and GAB2. However, a second more telomeric amplicon was also observed for which no candidate genes have been identified. In summary, amplification of these four putative oncogenes from 11q13 in early ovarian cancer is associated with a serous histology and in the case of EMSY and RSF1 a poor outcome. These findings support the hypothesis that the 11q13 amplicon in ovarian cancer is likely driven by a cassette of genes rather than by a single oncogene. This article contains Supplementary Material available at <http://www.interscience.wiley.com/jpages/1045-2257/suppmat>. (c) 2008 Wiley-Liss, Inc.

Press JZ, De Luca A, Boyd N, Young S, Troussard A, Ridge Y, Kaurah P, Kalloger SE, Blood KA, Smith M, **Spellman PT**, Wang Y, Miller DM, Horsman D, Faham M, Gilks CB, **Gray J**, Huntsman DG. Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer. 2008 Jan 22;8:17. PMID: 18208621

Subclassification of ovarian carcinomas can be used to guide treatment and determine prognosis. Germline and somatic mutations, loss of heterozygosity (LOH), and epigenetic events such as promoter hypermethylation can lead to decreased expression of BRCA1/2 in ovarian cancers. The mechanism of BRCA1/2 loss is a potential method of subclassifying high grade serous carcinomas. METHODS: A consecutive series of 49 ovarian cancers was assessed for mutations status of BRCA1 and BRCA2, LOH at the BRCA1 and BRCA2 loci, methylation of the BRCA1 promoter, BRCA1, BRCA2, PTEN, and PIK3CA transcript levels, PIK3CA gene copy number, and BRCA1, p21, p53, and WT-1 immunohistochemistry. RESULTS: Eighteen (37%) of the ovarian carcinomas had germline or somatic BRCA1 mutations, or epigenetic loss of BRCA1. All of these tumours were high-grade serous or undifferentiated type. None of the endometrioid (n = 5), clear cell (n = 4), or low grade serous (n = 2) carcinomas showed loss of BRCA1, whereas 47% of the 38 high-grade serous or undifferentiated carcinomas had loss of BRCA1. It was possible to distinguish high grade serous carcinomas with BRCA1 mutations from those with epigenetic BRCA1 loss: tumours with BRCA1 mutations typically had decreased PTEN mRNA levels while those with epigenetic loss of BRCA1 had copy number gain of PIK3CA. Overexpression of p53 with loss of p21 expression occurred significantly more frequently in high grade serous carcinomas with epigenetic loss of BRCA1, compared to high grade serous tumors without loss of BRCA1. CONCLUSION: High grade serous carcinomas can be subclassified into three groups: BRCA1 loss (genetic), BRCA1 loss (epigenetic), and no BRCA1 loss. Tumors in these groups show distinct molecular alterations involving the PI3K/AKT and p53 pathways.

Rizki A, Weaver VM, Lee SY, Rozenberg GI, Chin K, Myers CA, Bascom JL, Mott JD, Semeiks JR, Grate LR, **Mian IS**, Borowsky AD, Jensen RA, Idowu MO, **Chen F**, Chen DJ, Petersen OW, **Gray JW**, **Bissell MJ**. A human breast cell model of preinvasive to invasive transition. Cancer Res. 2008 Mar 1;68(5):1378-87. PMID: 18316601

A crucial step in human breast cancer progression is the acquisition of invasiveness. There is a distinct lack of human cell culture models to study the transition from preinvasive to invasive phenotype as it may occur "spontaneously" in vivo. To delineate molecular alterations important for this transition, we isolated human breast epithelial cell lines that showed partial

loss of tissue polarity in three-dimensional reconstituted basement membrane cultures. These cells remained noninvasive; however, unlike their nonmalignant counterparts, they exhibited a high propensity to acquire invasiveness through basement membrane in culture. The genomic aberrations and gene expression profiles of the cells in this model showed a high degree of similarity to primary breast tumor profiles. The xenograft tumors formed by the cell lines in three different microenvironments in nude mice displayed metaplastic phenotypes, including squamous and basal characteristics, with invasive cells exhibiting features of higher-grade tumors. To find functionally significant changes in transition from preinvasive to invasive phenotype, we performed attribute profile clustering analysis on the list of genes differentially expressed between preinvasive and invasive cells. We found integral membrane proteins, transcription factors, kinases, transport molecules, and chemokines to be highly represented. In addition, expression of matrix metalloproteinases MMP9, MMP13, MMP15, and MMP17 was up-regulated in the invasive cells. Using small interfering RNA-based approaches, we found these MMPs to be required for the invasive phenotype. This model provides a new tool for dissection of mechanisms by which preinvasive breast cells could acquire invasiveness in a metaplastic context.

Turley EA, Veisheh M, Radisky DC, **Bissell MJ**. Mechanisms of Disease: epithelial-mesenchymal transition—does cellular plasticity fuel neoplastic progression? Nat Clin Pract Oncol. 2008 Mar 18 PMID: 18349857

Epithelial-mesenchymal transition (EMT) is a phenotypic conversion that facilitates organ morphogenesis and tissue remodeling in physiological processes, such as embryonic development and wound healing. A similar phenotypic conversion is also detected in fibrotic diseases and neoplasia, and is associated with disease progression. EMT in cancer epithelial cells often seems to be an incomplete and bidirectional process. In this Review, we discuss the phenomenon of EMT as it pertains to tumor development, focusing on exceptions to the commonly held rule that EMT promotes invasion and metastasis. We also highlight the role of RAS-controlled signaling mediators, ERK1, ERK2 and phosphatidylinositol 3-kinase, as microenvironmental responsive regulators of EMT.